Please amend claims 1, 8, 9, 27, 66, 68, 71, 73 and 77-81 to read as follows:

1. (Amended) An isolated nucleic acid molecule encoding a NB-ARC and CARD containing protein (NAC), comprising a nucleotide sequence encoding a polypeptide having at least 80% identity to SEQ ID NO:4 or SEQ ID NO:6, or the complement of said nucleotide sequence,

wherein said polypeptide forms a CARD domain fold,

wherein said polypeptide does not comprise amino acids 957-987 of SEQ ID NO:2, and

wherein said polypeptide associates with SEQ ID NO:2 or with Apaf-1.

8. (Amended) An oligonucleotide consisting of at least 30 contiguous nucleotides up to 1035 contiguous nucleotides of the nucleotide sequence set forth in any of SEQ ID Nos: 1, 3 and 5 or the complement of said nucleotide sequence, said olignucleotide optionally having additional nucleotides at the 5' or 3' end that differ from the nucleotide sequence set forth in any of SEQ ID Nos: 1, 3 and 5 or the complement of said nucleotide sequence.

9. (Amended) The oligonucleotide of any one of claims 77, 78, 79, 80, 81 or 82, wherein said oligonucleotide is labeled with a detectable marker.

27. (Amended) A method for identifying nucleic acids encoding a mammalian NAC, said method comprising:

contacting a sample containing nucleic acids with the oligonucleotide of any one of claims 77, 78, 79, 80, 81 or 82, wherein said contacting is effected under high stringency hybridization conditions, and identifying compounds which hybridize thereto.

66. (Amended) A functional fragment of the nucleic acid molecule of either claim 1 or claim 71, wherein said functional fragment comprises a nucleotide sequence encoding a CARD domain corresponding to amino acids 1128-1261 and 1306-1473 of SEQ ID NO:2, and wherein said functional fragment associates with SEQ ID NO:2 or with Apaf-1.

68. (Amended) The nucleic acid molecule of claim 1, comprising a nucleotide sequence encoding amino acids 1128-1261 and 1306-1473 of SEQ ID NO:2.

71. (Amended) An isolated nucleic acid molecule encoding a NAC, comprising a nucleotide sequence encoding a polypeptide having at least 80% identity to SEQ ID NO:2, or the complement of said nucleotide sequence,

wherein said polypeptide comprises amino acids 1262-1305 of SEQ ID NO:2,

wherein said polypeptide forms a CARD domain fold, and

wherein said polypeptide associates with SEQ ID NO:2 or with Apaf-1.

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73. (Amended) The nucleic acid molecule of claim 71, comprising a nucleotide sequence encoding amino acids 1128-1261 and 1306-1473 of SEQ ID NO:2.

77. (Amended) An oligonucleotide consisting of the nucleotide sequence set forth as nucleotides 985-1641 of SEQ ID NO:1 or its complement, or a fragment thereof consisting of at least 20 contiguous nucleotides therefrom, said oligonucleotide or fragment optionally having nucleotides at the 5' or 3' end that differ from SEQ ID NO:1 or its complement.

78. (Amended) An oligonucleotide consisting of the nucleotide sequence set forth as nucleotides 2422-2844 of SEQ ID NO:1 or its complement, or a fragment thereof consisting of at

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least 20 contiguous nucleotides therefrom, said olignucleotide or fragment optionally having nucleotides at the 5' or 3' end that differ from SEQ ID NO:1 or its complement.

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79. (Amended) An oligonucleotide consisting of the nucleotide sequence set forth as nucleotides 3235-3960 of SEQ ID NO:1 or its complement, or a fragment thereof consisting of at least 20 contiguous nucleotides therefrom, said olignucleotide or fragment optionally having nucleotides at the 5' or 3' end that differ from SEQ ID NO:1 or its complement.

80. (Amended) An oligonucleotide consisting of the nucleotide sequence set forth as nucleotides 2870-2959 of SEQ ID NO:1 or its complement, or a fragment thereof consisting of at least 20 contiguous nucleotides therefrom, said olignucleotide or

fragment optionally having nucleotides at the 5' or 3' end that differ from SEQ ID NO:1 or its complement.

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81. (Amended) An oligonucleotide consisting of the nucleotide sequence set forth as nucleotides 4117-4419 of SEQ ID NO:1 or its complement, or a fragment thereof consisting of at least 20 contiguous nucleotides therefrom, said oignucleotide or fragment optionally having nucleotides at the 5' or 3' end that differ from SEQ ID NO:1 or its complement.

Please add new claims 87 and 88 as follows:

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87. (New) The oligonucleotide of any one of claims 77-81, wherein said fragment consists of at least 30 contiguous nucleotides.

88. (New) The oligonucleotide of claim 82, comprising at least 30 contiguous nucleotides.

REMARKS

Claims 1, 4-28 and 30-86 are pending. Non-elected claims 10, 12-17, 19-26, 28, 30-37 and 39-65 have been canceled without prejudice to pursuing the subject matter of these claims in one or more related applications claiming benefit of priority of the subject application. Claims 1, 8, 9, 27, 66, 68, 71, 73 and 77-81 have been amended. New claims 87 and 88 have been added. Following entry of the amendments, claims 1, 4-11, 27, 38 and 66-88 will be pending.

Claims 1 and 71 have been amended to indicate that the encoded NAC polypeptide forms "a CARD domain fold," which is an art-recognized term for the overall structural arrangement of the hexahelical CARD domains shown in Figure 1E (see, for example, Hofmann et al., Trends Biochem. Sci. 22:155-156 (1997) at page 156, column 2, which is incorporated into the specification at page 9, lines 10-12, and is attached hereto as Exhibit A).

Claim 8 has been amended to clarify that the oligonucleotide consists of at least 30 up to 1035 contiguous of the recited sequence. Claims 77-81 have been amended to clarify that the 20 contiguous nucleotides are a fragment of the longer recited portion. Claims 8 and 77-81 have also been amended to specify that the oligonucleotide or fragment can have different sequences at the 5' or 3' end. The amendment is supported, for example, at page 76, lines 6-18 where oligonucleotides containing NAC sequences with restriction sites at the 5' or 3' ends are described.

Claims 9 and 27 have been amended to remove reference to claim 8, and to specify that the claims are dependent on "any one of" the recited claims.

Claims 66, 68 and 73 have been amended to recite the amino acid boundaries of the $CARD_s$ domain. Support for the amendment can be found, for example, at page 78, lines 3-4.

New claims 87 and 88 specify that the fragment consists of, or the oligonucleotide comprises, respectively, at least 30

contiguous nucleotides. The amendments are supported, for example, at page 34, lines 7-9.

A marked-up version of the amended claims, showing the amendments with underlining and bracketing, is attached hereto as an Appendix.

The amendments herein adopt suggestions made by Examiner Beckerleg in a telephone interview conducted with Applicant's representative on January 28, 2002; place the claims into condition for allowance or remove issues for appeal; do not raise new issues for consideration; and do not introduce new matter. Accordingly, entry thereof is deemed to be proper and is respectively requested.

Applicant thanks Examiner Beckerleg for her time and helpful suggestions in a telephone interview with Applicant's representatives on January 28, 2002. The amendments and remarks herein are considered to be consistent with the discussions in that interview.

Rejections under 35 U.S.C. § 112, first paragraph

Written Description

Claims 1, 5-9, 11, 18, 27, 38, 66-69, 71-74 and 77-86 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking adequate written description. The rejection is respectfully traversed for the reasons that follow.

Applicant first notes that no grounds for rejecting claims 8, 9, 11, 27 or 77-82, relating to oligonucleotides with specified sequences, as allegedly lacking adequate written description have been raised. It is thus respectfully requested that the rejection with respect to these claims be removed.

With regard to claims 1, 5-7, 18, 38, 66-69, 71-74 and 83-86, which relate to nucleic acid molecules encoding a NAC polypeptide or CARD domain or NB-ARC domain fragment therefrom, the Action alleges that the specification does not provide adequate written description for the claimed nucleic acid molecules because first, it does not provide sufficient guidance as to any actual biological activity of the disclosed sequences, and second, the specification does not identify the actual physical, chemical and biological characteristics of these proteins so that it would be clear which sequences that vary from recited sequences would meet the claim limitations regarding biological activity. These points will be addressed in turn.

First, it is respectfully submitted that the specification provides sufficient guidance as to the required activity of the nucleic acid molecules recited in the claims. For example, independent claims 1 and 71 (and all claims dependent thereupon) require that the polypeptide encoded by the claimed nucleic acid molecule has the activity of associating with either SEQ ID NO:2 (NACβ) or with Apaf-1, which can be an *in vitro* or *in vivo* association. The specification teaches that a polypeptide that associates with either SEQ ID NO:2 or with Apaf-1 can be used in a variety of applications, including, for

example, in either *in vitro* or *in vivo* screening assays designed to identify agents that alter the association between SEQ ID NO:2 or Apaf-1 and their binding partners (page 49, line 16, to page 51, line 12). Agents identified by such a screen are expected to be effective in altering caspase activation in a cell or in altering the level of apoptosis in a cell, because of the established role of CARD domain containing proteins such as Apaf-1 in caspase activation and apoptosis. No further "biological activity" is required of the nucleic acid molecules of claims 1, 4-7, 66, 74-76 and 86.

Exemplary polypeptides that have the activity of associating with either SEQ ID NO:2 or with Apaf-1 are a fragment containing just amino acids 1128-1473 ($CARD_L$) of SEQ ID NO:2, a fragment containing just amino acids 1128-1261 and 1306-1473 ($CARD_s$) of SEQ ID NO:2 (recited in claims 66, 68 and 73, as amended), and a fragment containing just amino acids 326-551 (NB-ARC) of SEQ ID NO:2 (recited in claims 69, 74 and 86) (see, for example, page 78, lines 1-10 and Examples 3.0-6.0). Other exemplary polypeptides that have the activity of associating with either SEQ ID NO:2 or with Apaf-1 are SEQ ID NO:2 (recited in claim 75) and SEQ ID NOS:4 and 6 (recited in claim 70). As described further below, the skilled person would know, based on the guidance provided in the specification, which other NAC polypeptides have the activity of associating with either SEQ ID NO:2 or with Apaf-1.

Claims 38 and 83-85 further recite that the NAC polypeptide that associates with either SEQ ID NO:2 or with

Apaf-1 is expressed in a cell so as to modulate the level of apoptosis. As taught in the specification, a NAC polypeptide or fragment that associates with either SEQ ID NO:2 or Apaf-1 *in vitro* also associates *in vivo* (see Examples 3.0 to 6.0). As confirmed by Chu et al., J. Biol. Chem. 276:9329-9245 (2001), provided as Exhibit A with Applicant's Response mailed August 29, 2001, endogenous NAC and Apaf-1 can be co-immunoprecipitated from cells that intrinsically express NAC, using anti-NAC antisera, confirming that the association taught in the specification is biologically relevant (Chu et al. at page 9242, sentence bridging columns 1 and 2). As is well known in the art, Apaf-1 is a central activator of caspases. Expression of a NAC polypeptide encoded by a claimed nucleic acid molecule is thus expected to affect the activity of Apaf-1, thereby modulating the level of apoptosis in a cell.

Chu et al., supra (2001), further corroborates that expression of full-length NACβ (SEQ ID NO:2) sensitizes cells to suboptimal concentrations of the apoptotic inducer staurosporine and increases apoptosis when co-expressed with Apaf-1 and procaspase 9 (ie. modulates apoptosis positively). Chu et al. also corroborates that fragments of NAC (namely the CARD or NB-ARC domains of SEQ ID NO:2) interfere with apoptosis induced by staurosporine or by expression of Apaf-1 and procaspase 9 (ie. modulate apoptosis negatively). The skilled person appreciates that the ability to "modulate" the level of apoptosis in a cell encompasses the ability to enhance or inhibit apoptosis in the presence of apoptotic modulators routinely used in the art in apoptosis assays (e.g. staurosporine) or in cells that are

susceptible to apoptosis (e.g. many cancer cells, and cells that undergo apoptosis during development; such cells endogenously express high levels of Apaf-1). Therefore, Chu et al. corroborates the teachings in the specification that expression of NAC polypeptides according to claims 38 and 83-85 modulates the level of apoptosis in a cell.

Therefore, it is respectfully submitted that the specification provides sufficient guidance as to the required biological activities of the nucleic acid molecules recited in the claims.

Second, it is respectfully submitted that the specification also sufficiently identifies the physical, chemical and biological characteristics of the polypeptides encoded by the claimed nucleic acid molecules such that it would be clear which sequences that vary from the recited sequences meet the claim limitations regarding activity.

With regard to physical (structural) characteristics, claims 1 and 71, as amended, indicate that the polypeptide forms a CARD domain fold. At the time of the invention, the CARD domain, which is found in many proteins involved in apoptotic signaling, including Apaf-1, caspase-9, caspase-2, CED-4, CED-3, RAIDD, ICH-1, Mch6, Mch4, ICE, ICH-2, c-IAP1 and c-IAP2, was well known in the art to form a structurally conserved fold. The structures of the CARD domains of RAIDD and of wild-type and mutant Apaf-1, alone or in complex with the CARD prodomain of caspase-9, had been solved at high resolution (Qin et al., Nature

399:549-557 (1999), attached as Exhibit B and incorporated by reference into the specification at page 84, lines 31-32). CARD domains were thus known to be compact, globular structures with six (or seven) alpha helices surrounding a hydrophobic core. As corroborated by Chu et al., supra (2001), the CARD domain of NAC forms a similar fold as the CARD domains of Apaf1, pro-Casp9 and RAIDD (Figure 1E and page 9240, column 2 of Chu et al.).

The specification teaches, by overlining in Figure 1E, the particular residues of NAC that make up the alpha helical motifs that provide the characteristic CARD domain fold. Given the knowledge in the art regarding the secondary structure of alpha helices, the knowledge in the art regarding the tertiary structure of CARD domains, and the guidance in the specification regarding the specific residues that make up the alpha helices in NAC, the skilled person would have known which residues could be varied, and how they could be varied, while retaining the CARD domain fold.

With regard to chemical (sequence) characteristics, claims 1 and 71 recite that the polypeptide has at least 80% identity to the recited sequence. Thus, over the length of the polypeptide, up to 20% of the amino acids can be varied, so long as the recited structural features of the CARD domain (discussed above) and functional features (discussed below) are retained. The specification provides guidance as to preferred amino acid substitutions. For example, the specification teaches that conservative variations, such as substitution of a non-polar residue with another residue, or substitution of a charged

residue with a similarly charged residue, are recognized by those skilled in the art to not substantially alter the tertiary structure of the protein (page 31, lines 18-25). The specification also provides an alignment of the amino acid sequences of eight CARD domains (Figure 1E), with identical residues indicated, which provides further guidance regarding variations unlikely to alter the secondary or tertiary structure.

With regard to biological (functional) characteristics, claims 1 and 71 recite that the polypeptide associates with either SEQ ID NO:2 or with Apaf-1. As taught in the specification, a CARD domain fragment containing residues 1128-1261 and 1306-1473 of SEQ ID NO:2 is sufficient for the activity of self-associating or associating with Apaf-1. Likewise, a NB-ARC domain fragment containing residues 326-551 of SEQ ID NO:2 is sufficient for the activity of self-associating. Accordingly, the skilled person would predict that essentially any sequence variations in a NAC polypeptide outside of these minimal regions would not affect the recited activity. As described above with regard to structural features, the skilled person could also predict which sequence variations within the CARD domain would not affect associating activity.

In view of the above amendments and remarks, it is respectfully submitted that the specification provides adequate written description commensurate in scope with the claims.

Enablement

Claims 1, 5-9, 11, 18, 27, 38, 66-69, 71-74 and 77-86 also stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking adequate enablement for the full scope of the claims. The rejection is respectfully traversed for the reasons that follow.

Applicant first notes that no grounds for rejecting claims 8, 9, 11, 27 or 77-82, relating to oligonucleotides with specified sequences, as allegedly lacking adequate enablement have been raised. It is thus respectfully requested that the rejection with respect to these claims be removed.

With regard to the remaining claims, the Action notes that the specification demonstrates "*in vitro* protein:protein interactions between the novel NB-ARC and CARD domains of the instant invention and several taught by the art," but alleges that the specification "does not provide any guidance as to any specific biological activity of the novel "NAC" proteins encoded by the disclosed cDNA or demonstrate that any of the disclosed proteins or protein domains or fragments have any apoptosis modulating activity either *in vitro* or *in vivo*."

As described above with regard to written description, independent claims 1 and 71 only require that the polypeptide encoded by the claimed nucleic acid molecule has particular structural and sequence characteristics, and the activity of associating with SEQ ID NO:2 or Apaf-1. Claims 38 and 85-87

further require that the polypeptide encoded by the claimed nucleic acid molecule be expressed so as to modulate apoptosis. It is respectfully submitted that the specification provides sufficient guidance such that the skilled person could have made and used the claimed invention without undue experimentation.

As taught in the specification, a polypeptide that "associates" is a polypeptide that binds to a protein relatively specifically and, therefore, can form a bound complex *in vivo* in a cell or *in vitro* under suitable conditions (page 14, lines 15-24). Suitable assays for determining *in vivo* or *in vitro* association of a full-length NAC or a NAC fragment with SEQ ID NO:2 or with Apaf-1, including two-hybrid assays, affinity column assays, GST-pull-down assays, expression screening assays and co-immunoprecipitation assays, are described, for example, at page 71, line 31 to page 74, line 2, and in Examples 3.0-6.0 (pages 78-84). Suitable conditions for conducting such assays are also described at page 54, lines 20-32. Given these teachings, and the guidance described above regarding the residues important for NAC structure and function, it is respectfully submitted that it would not have required undue experimentation to confirm that a particular NAC polypeptide, having the recited structural and sequence properties, retains the recited associating activity, and thus it would not have required undue experimentation to make and use the invention of claims 1, 71 and their dependents.

Likewise, to confirm that a particular variant NAC polypeptide retains the ability to modulate apoptosis, the skilled person could have performed any of the apoptosis assays

routinely used in the art at the time of the invention, including DNA morphological assays such as those described in Chu et al. supra by reference to 1997 and 1998 publications. In performing such assays, the skilled person would have chosen cells that are sensitive to apoptosis, including cancer cells, cells that developmentally undergo apoptosis, cells that overexpress apoptosis modulators (such as Apaf-1/caspase-9) and cells treated with apoptosis inducers, such as staurosporine. It is thus respectfully submitted that it would not have required undue experimentation to confirm that a particular NAC polypeptide modulates the level of apoptosis in a cell, and thus it would not have required undue experimentation to make and use the invention of claims 38 and 85-87.

Roth et al. (Exhibit B of Applicant's Response mailed August 29, 2001) was previously submitted to corroborate that a variety of vectors can be used to predictably express apoptosis-modulating genes *in vivo*. However, the Action alleges that a nexus cannot be drawn between the teachings of Roth et al. and the claimed methods.

The Action states that NAC and p53 do not share any biological functions. However, like NAC, p53 also has the ability to modulate apoptosis when expressed in cells (see, for example, page 149, column 2, paragraph 2 of Roth et al. which states that "p53 plays a central role in...control of apoptosis," and page 152, column 3, paragraph 3 which states that "TUNEL staining...of the posttreatment biopsy samples showed that

apoptosis had increased following treatment [with a retroviral vector expressing p53]).

The Action quotes a sentence from the *introduction* of Roth et al. (page 148, column 1) as indicating that gene therapy of disease was not considered predictable in 1999. However, this statement is clearly directed to "disease[s] caused by the absence or mutation of a single gene, such as cystic fibrosis or Gaucher's disease," and the difficulty in curing or treating such diseases by "inserting a normal copy of the mutant or deleted gene into a renewable population of host cells, such as bone marrow stem cells." Claims 38 and 85-87 do not recite, and enablement of these claims does not require, long term expression of the gene or cure or treatment of a disease. It is respectfully submitted that the remainder of the Roth et al. publication describes a variety of methods that have achieved successful delivery and expression of an apoptosis modulating gene in cells so as to modulate apoptosis in a cell.

Furthermore, the Action also appears to indicate that Roth et al. only teaches adenoviral gene therapy. However, Roth et al. states, based on clinical trial results, that "...retroviral vectors yield levels of gene expression sufficient to demonstrate a biological effect" (page 149, column 2).

Accordingly, Applicant maintains, for the reasons of record, that Roth et al. provides corroboration for the predictability of the claimed methods.

In summary, in view of the above remarks and amendments, it is respectfully submitted that the specification provides adequate written description and enablement for the claimed invention and, therefore, reconsideration and removal of the rejection is respectfully requested.

Claim Objections

Claims 9 and 27 are objected to under 37 C.F.R. § 1.75(c) as allegedly being in improper form. Claims 9 and 27 have been amended as suggested in the Action to obviate this rejection. Accordingly, removal of the objection is respectfully requested.

Rejections under 35 U.S.C. § 102

Claims 8 and 77-82 stand rejected under 35 U.S.C. § 102(a) as allegedly anticipated by Nagase et al. (1999). The rejection is traversed for the reasons that follow.

The Action alleges that the language "comprising," as used in the rejected claims, opens the claims up to encompass the sequence described by Nagase et al. (1999). Claims 8 and 77-81 have been amended to recite the closed term "consisting of" with regard to fragments of the recited sequence, while maintaining that the oligonucleotide can optionally have, at the 5' or 3' end, additional sequences that differ from the recited sequence. Such additional sequences can include, for example, restriction

sites and the like that are commonly appended to oligonucleotides (see, for example, the PCR primers recited in Example 1.0).

Claim 82 recites an oligonucleotide comprising at least 20 contiguous nucleotides of the nucleotide sequence set forth as nucleotides 3784-3915 of SEQ ID NO:1 or its complement. Nucleotides 3784-3915 of SEQ ID NO:1 correspond to exon 2 of NACβ (see Figure 1B and 1C), a region not present in the Nagase et al. (1999) sequence. Accordingly, it is respectfully submitted that the rejection over Nagase et al., (1999), should not be applied to claim 82.

In view of the above amendments and remarks, reconsideration and removal of the rejection under 35 U.S.C. § 102(a) is respectfully requested.

Rejection under 35 U.S.C. § 103(a)

Claims 9, 11 and 27 stand rejected under 35 U.S.C. § 103(a) as allegedly obvious over Nagase et al. (1999) in view of Nagase et al. (1998). Applicant understands from the interview held on January 28, 2002, that the rejection of claims 9, 11 and 27 applies only insofar as they depend on claim 8, because it was acknowledged that the combination of references does not teach or suggest oligonucleotides from the particular regions recited in claim 77-82. Claims 9 and 27, as amended, and thus claim 11, which depends on claim 9, no longer depend on claim 8.